

# Complete Mitochondrial Genome Sequences for Four Bryozoan Species

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## Abstract

Based on shared morphological characteristics, bryozoans have traditionally been grouped with phoronids and brachiopods to form the Lophophorata, a superphylum within the deuterostomes. However, molecular comparisons (mainly from 18S phylogenies) have challenged this placement suggesting that bryozoans are protostomes of the lophotrochozoan clade. There are enough lophotrochozoan genomes now available in the databases for comparative genomic analysis. Thus, to further test this phylogenetic hypothesis we sequenced complete mitochondrial genomes for the ectoprocts: *Bugula* sp., *Flustrellidra* sp., *Membranipora* sp. and *Electra* sp. These mitochondrial genomes are relatively small (approximately 14 kb) when compared to other metazoans, and the gene arrangements are very divergent among them as well as when compared to other lophotrochozoan taxa. We will be presenting the results from both gene order comparisons and protein encoding genes for phylogenetic reconstruction to test for the monophyly of bryozoans and lophophorates within the Lophotrochozoa.

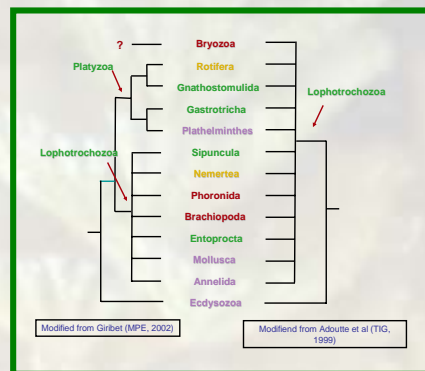


Figure 1

Contending phylogenetic hypotheses for the phyla belonging to the Lophotrochozoa

## Introduction

The bryozoans or moss animals are sessile colonies of zooids living in marine and freshwater environments. There are three classes of bryozoans: the Gymnolaemata, Stenolaemata and the Phylactolaemata (Brusca and Brusca 2002). Classical taxonomy unites the bryozoans with the brachiopods and phoronids to form the Lophophorata. All lophophorates are characterized by the presence of a ring of ciliated tentacles, the lophophore, emerging from the mesosome, which gives the group its name. The lophophorates were originally believed to be deuterostomes due to the radial cleavage, enterocoelic formation of the coelom, and the development of the mouth from a secondary opening. However molecular studies have challenged this hypothesis (Halanych et al. 1995, Aguinaldo et al. 1997). Small subunit sequences (SSU) suggest that bryozoans, along with phoronids, brachiopods and platyhelminthes are protostomes and more particularly lophotrochozoans. For this preliminary analysis, we have sequenced the complete mitochondrial genome of the bryozoans *Membranipora* sp., *Flustrellidra* sp., *Electra* sp. and *Bugula* sp., and a partial genome of *Schizoporella* sp., which are all within the class Gymnolaemata. The goal of this preliminary analysis is to elucidate whether the bryozoans as well as the lophophorates are monophyletic groups and their phylogenetic status within lophotrochozoans.

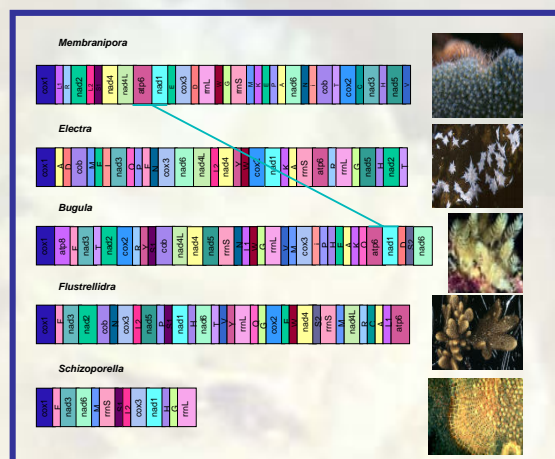


Figure 2

The bryozoan mitochondrial genomes sequenced present highly rearranged gene orders with respect to one another, as well as with respect to other metazoans.

## Materials and Methods

The bryozoan genomic DNA was extracted using the DNeasy Tissue Extraction Kit from Qiagen. The LA-Taq Kit was used to amplify half of the mitochondrial genome using primers matching conserved regions. Species-specific primers were then designed using Oligo 4.0. to amplify the second half of the circular molecule. Shotgun DNA libraries were constructed from gel-purified PCR products followed by sequencing of randomly chosen clones. Sequence assembly was done using Phrap and visualized in Consed and Sequencher. Genome annotation was done using Mitotator (inhouse software), Dogma (<http://bugmaster.jgi-psf.org/dogma>) and tRNAscan (<http://www.genetics.wustl.edu/eddy/tRNAscan-SE/>). Maximum Parsimony trees were obtained using PAUP 4.0 using 12 genes (Atp6, Cob, Cox1, Cox2, Cox3, Nad1, Nad2, Nad3, Nad4, Nad4L, Nad5, and Nad6). Minimum evolution trees were obtained using MEGA 2.0.

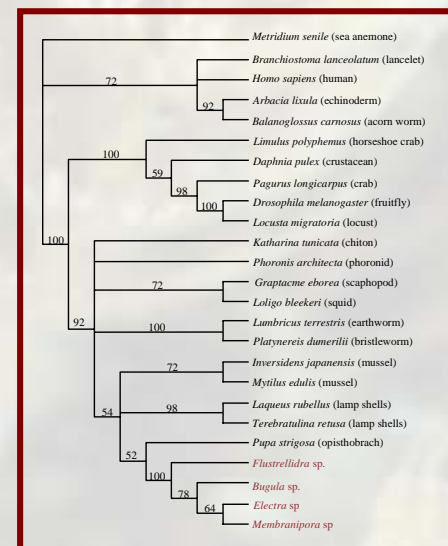


Figure 3

Maximum Parsimony tree showing that the sampled bryozoans (red) are monophyletic. The Lophophorata is polyphyletic in this tree, although with weak support, since the relevant branches have bootstrap values of only 52 and 54.

## Results and Discussion

The four complete mitochondrial genomes of the bryozoans are similar in size. The lengths of these genomes is: *Bugula* 14,911 bp, *Flustrellidra* 13,052 bp, *Membranipora* 14,750 bp and *Electra* 13,633 bp. In addition to the latter we were also able to successfully sequence a 6 kb fragment of *Schizoporella* sp.

For this study we executed a preliminary Maximum Parsimony (MP) analysis that yielded one most parsimonious tree, in which the lophotrochozoans are monophyletic and supported with a high bootstrap score of 92. We also confirmed that the gymnolaemate bryozoans are monophyletic, with a bootstrap score of 100. To verify if bryozoans are a monophyletic group more data from the classes of Stenolaemata and Phylactolaemata would be essential. On the other hand, Lophophorata may be polyphyletic, although the support here is low. It seems clear that more data is needed to obtain better resolution within the Lophotrochozoa.

There is almost no similarity among the mitochondrial gene arrangements of these gymnolaemate bryozoans, a degree of rearrangement previously found in some mollusks. We intend to do future work to determine whether this is a feature uniquely of this clade or characterizes the Bryozoa more broadly.

